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Evolutionary ecology of opsin gene sequence, expression and repertoire.

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Linking molecular evolution to biological function is a longstanding challenge in evolutionary biology. Some of the best examples of this involve opsins, the genes that encode the molecular basis of light reception. In this issue of Molecular Ecology, three papers examine opsin gene sequence, expression and repertoire to determine how natural selection has shaped the visual system. Firstly, Escobar-Camacho *et al.* (2017) use opsin repertoire and expression in three Amazonian cichlid species to show that a shift in sensitivity towards longer wavelengths is coincident with the long wavelength dominated Amazon basin. Secondly, Stieb *et al.* (2017) explore opsin sequence and expression in reef-dwelling damselfish and find that UV- and long-wavelength vision are both important, but likely for different ecological functions. Lastly, Suvorov *et al.* (2017) study an expansive opsin repertoire in the insect order Odonata and find evidence that copy number expansion is consistent with the permanent heterozygote model of gene duplication. Together these papers emphasize the utility of opsin genes for studying both the local adaptation of sensory systems and, more generally, gene family evolution.

Opsin genes: a playground for evolutionary ecologists.

Opsins, which are g-protein coupled receptors, are the molecular basis of colour vision. A subset of opsin proteins, while bound to chromophores, absorb light to initiate the visual transduction cascade that eventually results in the perception of light. Visual opsins, a subset of opsins genes responsible for vision, have considerable variation in repertoire size among taxa ranging from fifteen in invertebrates such as stomatopods, to ten for many fish and three for most mammals (Porter *et al.* 2009; Rennison *et al.* 2012; Jacobs 2009). Each opsin protein is sensitive to a particular range of wavelengths and this range is controlled by the amino acid sequence (Yokoyama 2000). Previous research has tested and described the effects of individual coding-sequence mutations on wavelength sensitivity. This allows the phenotype of opsin gene proteins to be predicted from nucleotide sequences alone (Yokoyama 2000). As colour vision is thought to be under strong natural and sexual selection in many species (*e.g.* Endler 1991) due to the role it plays in predator avoidance, social interactions, foraging, and mate choice, the direct and well characterized connection between opsin gene sequence and colour vision make these genes especially amenable to studies of adaptive evolution. The studies highlighted here help to illuminate the different mechanisms that create and maintain opsin gene sequence and repertoire diversity (Figure 1).

Seeing red: multiple genetic mechanisms contribute to local adaptation.

The first study by Escobar-Camacho *et al.* (2017) characterized the molecular basis of vision in three species of Amazonian cichlid using next generation sequencing of whole genomes and retinal transcriptomes (Figure 2A). The authors looked for evidence of adaptation to a murky riverine environment and contrasted the patterns of differentiation with those of the well-studied African cichlids (*e.g*. Carleton and Kocher 2001).

Escobar-Camacho *et al.* find that compared to their African relatives, Amazonian cichlids have undergone changes in their opsin gene repertoires, expression profiles, and coding sequences. The changes in gene expression and coding sequence have shifted the spectral capabilities of Amazonian cichlids towards longer wavelengths. These shifts qualitatively matched the spectral environment of the Amazon basin, which is dominated by long-wavelengths, suggesting that the changes were adaptive. Furthermore, Amazonian cichlids exhibited a pattern of repeated pseudogenization of shorter wavelength-sensitive opsin genes, which suggests that these gene losses may be adaptive rather than due to drift. Similar patterns of gene loss and shifts in maximal absorbance have been shown to be common for deep-sea fish, which also live in light limited environments (Reviewed by Davies *et al.* 2012).

Most strikingly, Escobar-Camacho *et al.* show that mechanistically, the diversification of opsins in Amazonian cichlids has arisen due to a combination of gene duplication, gene conversion, gene loss and novel mutation events. This finding provides further support to previous work, which has suggested that the evolution of opsin genes is a rapid (*e.g*. Rennison *et al.* 2016) and dynamic process (*e.g.* Cortesi *et al.* 2015) where multiple molecular processes contribute to the generation of novel spectral phenotypes and local adaptation (Carleton et al., 2016).

The long and short of it: ecologically driven changes in spectral tuning.

The second paper by Stieb *et al.* (2017) examined patterns of opsin sequence divergence and gene expression for a range of damselfish species from Australia's Great Barrier Reef (Figure 2B). The authors first looked for evidence of natural selection on spectral tuning sites and then asked whether ecological variables explained the patterns of gene expression and sequence divergence.

Closely related taxa are often found in vastly different spectral environments and previous work from a variety of species has shown that organisms can alter their visual systems in response to these divergent spectral conditions (reviewed by Hunt e*t al.* 2009). The damselfish of the Great Barrier Reef are generally found in shallow broad-spectrum environments. However, they exhibit tremendous ecological and morphological diversity (Allen 1991), which may require a unique visual ability for each habitat or ecological niche. Previous work in this system suggested that some opsin genes were under positive selection during the adaptive radiation of damselfish (Hofmann *et al.* 2012). Remarkably Stieb *et al.* demonstrate that selection has remained strong among closely related species which suggests that colour vision may play an important role in diversification, as has been suggested for other taxa (*e.g.* Seehausen *et al.* 2008). Among these species patterns of evolution varied between parallel, divergent and reverse, indicating that selection pressures may differ among the taxa.

Stieb *et al.* found that the greatest divergence, even among close relatives, occurred in short-wavelength (*SWS1* and *SWS2B*) and long-wavelength (*LWS*) sensitive opsins. This finding contrasts work in many other systems, which has shown that middle and long-

wavelength opsins often exhibit considerably more sequence diversification than shortwave opsins (*e.g.* Spady e*t al.* 2005). Functionally, Stieb *et al.* suggest that UV-perception and -signals may be used as private channel of communication given that most predatory reef fish lack the capacity for UV perception (Marshall and Cheney 2011). This is supported by evidence in songbirds and other fish species suggesting that UV wavelengths can be used as a private signalling channel (e.g. Cummings et al. 2003). Stieb *et al.* also find that a higher level of *LWS* expression is associated with a more herbivorous diet.This suggests that multiple ecological functions shape and perhaps constrain patterns of spectral divergence in this radiation.

When more is better: permanent heterozygote advantage from opsin gene duplication.

The third study by Suvorov *et al.* (2017) explores the evolutionary model behind a remarkable case of opsin gene repertoire expansion. Although fish are known for having some of the largest opsin repertoires among vertebrates, odonates (dragonflies and damselflies) have even larger opsin copy numbers, up to 30 genes (Futahashi *et al.* 2015) (Figure 2C). This begs the question, why would so many gene duplicates become fixed?

Suvorov *et al.* attempt to answer this question by tapping into the robust theory on gene-duplication evolution. Currently, four categories of models of gene duplication exist. Briefly, duplicates can fix neutrally and then later evolve a function (Category I); duplication itself can be adaptive (Category II); duplicates can be immediately adaptive and have a function (Category III); lastly, duplicates may fix gene dosage imbalances (Category IV) (Innan and Kondrashov 2010). These categories, and the models within them, have different predictions for if and when positive, negative and neutral selection patterns are expected to occur during gene evolution. To determine which model best fit odonate opsin evolution, the authors used PAML and a novel Bayesian “diffusion” model of selection that parses positive selection into pre- and post-duplication phases (Yang 2007). They find that positive selection primarily occurred pre-duplication. Together with the reasonable hypothesis that new opsin duplicates are evolving under a subfunctionalization trajectory, their data supports the permanent heterozygote model of gene duplication (Category III). This model suggests that there is heterozygote advantage and positive selection for diverse opsin alleles before duplication and that gene duplication then fixes divergent alleles in the genome. This work provides a compelling step forward towards explaining large opsin gene repertoires as well as a new method for testing hypotheses in other systems.

Prospects and Conclusions.

Several themes repeatedly emerged from these three studies and inform us on the processes shaping the evolutionary trajectories of opsin genes.

The work of Stieb *et al.* (2017) demonstrates spectral tuning via structural and expression changes, whereas Escobar-Camacho *et al.* (2017) show variation in opsin gene repertoire, structure and expression. Both studies reinforce the idea that the local environment plays an important role in shaping the evolution of the visual system, whether it be the wavelengths most common in the environment or the ecological tasks to be performed. However, shifts in opsin gene expression can be both evolutionary and plastic depending on the species and circumstance. Further work will be required to determine whether the shifts in opsin gene expression found by Escobar-Camacho *et al.* and Stieb *et al.* are the result of evolutionary

changes, phenotypic plasticity or both. Quantitative trait locus mapping of expression differences (eQTL) (*e.g.* O'Quin *et al*. 2012) could also be a useful technique for furthering our understanding of the genetic basis of adaptation to differential spectral conditions in these (and other) systems.

The work by Suvorov *et al.* provides a critical first step towards explaining the expansive opsin gene repertoires found in many taxa. In tamarins and other primates, females heterozygous for LWS opsin genes have an advantage detecting ripe fruit but it remains to be seen whether enhanced colour vision plays any role in odonate evolution (Smith *et al.* 2003). Further work needs to be done to determine if the heterozygote advantage model is common for opsin gene expansion in other taxa and to determine the functional divergence between new duplicates that selection is acting on.

All three papers demonstrate the fluidity of opsin gene repertoire, specifically in terms of gene loss or pseudogenization. Most current models of opsin gene loss emphasize gene loss as a neutral process when the wavelengths of light that an opsin is sensitive to are no longer available (*e.g.* marine mammals (Newman and Robinson 2005)). An alternative hypothesis is that gene loss is adaptive; for example if additional opsin expression reduced target sensitivity or decreased colour discrimination, gene loss may be directly favoured. This idea could more easily be explored in taxa like odonate or percomorph fishes with large and labile opsin gene repertoires.

While current research, including the above papers, strongly implicates a role for selection in shaping the evolutionary trajectories of opsins, much of the evidence is correlational. Direct measures of natural and sexual selection on opsin genes are still required to affirm this notion. Furthermore, functional work linking genotype to phenotype has largely been undertaken *in vitro*, estimates of phenotypic effects now need to be conducted using behavioural assays and proteomic techniques to confirm that changes in gene expression and sequence have biologically relevant effects.

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Figures

Figure 1. Processes that create opsin gene and repertoire diversity. Gene duplication creates new opsin genes (A), sequence divergence changes opsin properties (B), pseudogenization removes functional opsin genes (C), regulatory changes modify opsin gene expression (D) and partial gene conversion creates new mutation combinations (E).

Figure 2. Example taxa for Amazonian cichlids (*Astronotus ocellatus*) (A), damselfish (*Chromis viridis*) (B), and odonates (*Gomphus vulgatissimus*) (C). Photos by Jón Helgi Jónsson, Steve Parrish, and Dariusz Kowalczyk respectively.